I hereby certify that this correspondence is being submitted to the United States Patent and Trademark Office via EFS on Sept 28, 2010.

Gail Wardwell

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re application of:

E. Raschke *et al.*

Application No.: 09/844,662

Filed: April 27, 2001

For: METHODS FOR BINDING AN EXOGENOUS MOLECULE TO CELLULAR CHROMATIN

Examiner: Robert M. Kelly

Group Art Unit: 1633

Confirmation No.: 9004

**REPLY BRIEF**

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This Reply Brief is responsive to the Examiner's Answer mailed August 3, 2010. As a Reply Brief can be filed on or before October 3, 2010, this Brief is timely filed.

## REAL PARTY IN INTEREST

Sangamo BioSciences, Inc. is the assignee of record, based on an assignment from the inventors recorded on October 16, 2001 at Reel 012068, Frame 0811. Thus, Sangamo BioSciences, Inc. is the real party in interest.

## RELATED APPEALS AND INTERFERENCES

Other than the Appeals in cases in which Sangamo BioSciences is the Real Party in Interest as listed in the Appeal Brief, Appellants are not aware of any other Related Appeals or Interferences.

## STATUS OF CLAIMS

Pending: Claims 57, 68-71, 87-91 and 96-102

Canceled: Claims 1 to 56, 58-67, 72-90, 92-95

Withdrawn: Claims 91, 93, and 96-102

Rejected: Claims 57 and 68-71

Appealed: Claims 57 and 68-71

## GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

**A.** Whether claims 57 and 68-71 are unpatentable under 35 U.S.C. § 112, 1st paragraph (written description) for containing new matter not disclosed in the as-filed specification.

**B.** Whether claims 57 and 68-71 are unpatentable under 35 U.S.C. § 112, 1st paragraph (written description) for containing matter not disclosed in the as-filed specification.

**C.** Whether claims 57, 68, 70 and 71 are unpatenable 35 U.S.C. § 102(e) as anticipated by U.S. Patent No. 6,013,453 (hereinafter "Choo").

**D.** Whether claims 57 and 68-71 are unpatentable under 35 U.S.C. § 103(a) as obvious over Choo in view of WO 00/9837755 (hereinafter "Dangl").

## ARGUMENTS

### A. The claims on appeal do not contain new matter

Claims 57 and 68-71 remain rejected under 35 U.S.C. § 112, 1st paragraph allegedly containing new matter not described in the originally filed specification, namely a non-naturally occurring zinc finger protein comprising at least one zinc finger domain with a non-naturally occurring recognition helix that binds to a target site in region of chromosomal cellular chromatin that is sensitive to DNAseI digestion. (Examiner's Answer, pages 6-7). The Examiner's Answer addresses the rejection under 35 U.S.C. § 112, 1st paragraph in two parts: a "new matter alone" rejection and a "new matter and written description rejection." *Id.*

In terms of the "new matter alone" rejection, the Examiner asserts that "no Artisan could reasonable envision" that the use of DNAse I digestion to be part of the invention. *Id.* at page 6.

The Examiner errs in asserting that the specification as filed does not fully describe binding of zinc finger proteins to regions of chromatin that are sensitive to digestion with DNAseI (*see, e.g.,* page 13, lines 20-26; and Example 15 beginning on page 38, bolding added):

> **In one embodiment, an enzymatic probe of chromatin structure is used to identify an accessible region. In a preferred embodiment, the enzymatic probe is DNase I (pancreatic deoxyribonuclease).** Regions of cellular chromatin that exhibit enhanced sensitivity to digestion by DNase I, compared to bulk chromatin (*i.e.*, DNase-hypersensitive sites) are more likely to have a structure that is favorable to the binding of an exogenous molecule, since the nucleosomal structure of bulk chromatin is generally less conducive to binding of an exogenous molecule. Furthermore, DNase-hypersensitive regions of chromatin often contain DNA sequences involved in the regulation of gene expression.
>
> *Design of a ZFP targeted to an accessible region of the ER-α gene*
>
> An engineered fusion protein was designed to recognize a unique 9 base pair sequence in the **DNase I hypersensitive region** at –2 kb. This protein (BOS 3) comprised a nuclear localization sequence, a zinc finger binding domain, a KRAB repression domain and a FLAG epitope. The zinc finger binding domain was targeted to the sequence GGGGAGGAG, (SEQ ID NO: 27) which is complementary to the sequence CTCCTCCCC (SEQ ID NO: 28) in the coding strand. Zinc finger sequences (for amino

acids –1 through +6 of the recognition helices) were RSDNLTR (SEQ ID NO: 29), RSDNLTR (SEQ ID NO: 30) and RSDALTK (SEQ ID NO: 31). Construction of a plasmid encoding the fusion protein and determination of the binding affinity of the zinc finger binding domain for its target sequence were performed according to methods disclosed in co-owned PCT WO 00/41566 and WO 00/42219. The dissociation constant (Kd) was determined to be 3.5 pM.

*Assay for binding of designed ZFPs*

....

The results, shown in Figure 5, show an approximately 20-fold enrichment of ER-alpha sequences associated with BOS3 in MCF-7 cells, compared to MCF-7 cells in which BOS3 was not expressed. **Thus, chromatin immunoprecipitation indicates that an exogenous molecule, targeted to an accessible region of cellular chromatin, binds to its target site *in vivo*.**

Clearly then, the original specification amply describes DNAseI digestion to identify accessible regions of cellular chromatin.

Furthermore, it is axiomatic that the original claims are part of the disclosure and do not constitute new matter. In the case on appeal original claims 40, 30 and 33-36 clearly describe the claims on appeal:

**40.** A complex between an exogenous molecule and a binding site, wherein the binding site is located within a region of interest in cellular chromatin and wherein the binding site is identified according to the method of claim 30.

**30.** A method for identifying a binding site for an exogenous molecule, wherein the binding site is located within a region of interest in cellular chromatin, wherein the method comprises:

(a) identifying an accessible region within the region of interest; and

(b) identifying a target site for the exogenous molecule within the accessible region.

**33.** The method according to claim 30 wherein the accessible region is a nuclease hypersensitive region.

**34.** The method according to claim 30 wherein the exogenous molecule is a protein.

**35.** The method according to claim 34 wherein the protein is a transcription factor.

**36.** The method according to claim 35 wherein the transcription factor is a ZFP.

Thus, the Examiner's rejection is without merit. The as-filed specification, including original claims, explicitly describes the claimed subject matter, namely a cell in which a 3 fingered non-naturally occurring zinc finger protein is bound to target site in a region of cellular chromatin that is sensitive to digestion with DNAseI. Accordingly, the rejection cannot be sustained.

**B. The claims on appeal are amply described by the as-filed specification**

The Examiner has also maintained that rejection that the term "non-naturally occurring" was unclear, undefined and not described in the as-filed specification. (Examiner's Answer, pages 7-8).

In response to Appellants noting their attempt to advance prosecution by clarifying that the zinc finger proteins as a whole are non-naturally occurring by virtue of the non-naturally occurring recognition helices of the individual zinc fingers, the Examiner stated that "further prosecution is not allowed" after final. (Examiner's Answer, page 11).

As a threshold matter, it is noted that the Examiner errs in asserting that further prosecution is not allowed after final. In fact, amendments after final can be entered at the discretion of the Examiner, for example to clarify or to simplify the issues for appeal (37 C.F.R. § 1.116(b)):

> (b) After a final rejection or other final action (§ 1.113) in an application or in an ex parte reexamination filed under § 1.510, or an action closing prosecution (§ 1.949) in an inter partes reexamination filed under § 1.913, but before or on the same date of filing an appeal (§ 41.31 or § 41.61 of this title):
>
> (1) An amendment may be made canceling claims or complying with any requirement of form expressly set forth in a previous Office action;

> (2) An amendment presenting rejected claims in better form for consideration on appeal may be admitted; or
>
> (3) An amendment touching the merits of the application or patent under reexamination may be admitted upon a showing of good and sufficient reasons why the amendment is necessary and was not earlier presented.

As the proposed amendments were clarifying and simplified the issues for appeal, the Examiner could have entered them. There is no proscription against Examiners entering amendments after final.

Furthermore, in response to Appellants' arguments that the skilled artisan was well aware that the term "non-naturally occurring" in the context of zinc fingers meant a protein with at least one altered recognition helix, the Examiner stated (Examiner's Answer, page 12 and page 14):

> Such is not persuasive. The rejection is based on the presence of 3 zinc fingers being present, and that one of the fingers contain a non-naturally occurring recognition helix, and it is bound to a region which is sensitive to DNAseI. The genera excludes less than 3 fingers ... Appellant's disclosure as originally filed does not provide sufficient disclosure to evidence possession of such generic embodiment. What Appellant is attempting to get at is a claim to those that are designed or selected ...
>
> To recap, the basis of the rejection is to a generic finger, with three fingers or more, one of which contains a non-naturally occurring recognition, and bound to a region which is sensitive to DNAseI digestion.

First and foremost, the Examiner errs in asserting that the claims encompass zinc finger proteins of 3 or more zinc fingers in which "only one of which [fingers] contains" a non-naturally occurring recognition helix. Claim 57 (from which all the claims on appeal depend) clearly states that each and every zinc finger domain is non-naturally occurring (see, Claims Appendix, claim 57, emphasis added):

> **57.** A cell comprising a complex between
>
> a non-naturally occurring zinc finger protein comprising 3 or more zinc finger domains, <u>wherein the zinc finger domains comprise a non-naturally occurring recognition helix</u> and
>
> chromosomal cellular chromatin;

> wherein the zinc finger protein is bound to a target site in a region of the cellular chromatin that is sensitive to digestion with DNAseI.

Thus, it is erroneous to assert that Appellants' claims are directed to zinc finger proteins in which only one component zinc finger is non-naturally occurring. Rather, the claims clearly state that all zinc fingers comprise a non-naturally occurring recognition helix region.

Furthermore, it remains the case the as-filed specification clearly describes the claimed subject matter, namely zinc finger proteins that are non-naturally occurring by virtue of having designed or selected recognition helices. *See,* page 5, lines 14-25 (paragraph [0019] of published application); page 6, lines 20-31 (paragraph [0025] of the published application); page 17, lines 4-20 (paragraph [0070] of published application); Example 8 (paragraphs [0110], [0111] and [0114] of published application); Table 1 which shows exemplary non-naturally occurring zinc finger recognition domains; Example 15 (paragraph [0158] of published application), emphasis added):

> In another embodiment, an accessible region is identified within a region of interest and a ZFP target site is located within the accessible region. A ZFP that binds to the target site is designed. The designed ZFP can be introduced into the cell, or a nucleic acid encoding the designed ZFP can be designed and the designed nucleic acid can be introduced into the cell, where it will express the designed ZFP. Methods for the design and/or selection of ZFPs that bind specific sequences are disclosed in U.S. Pat. No. 5,789,538; U.S. Pat. No. 6,007,408; U.S. Pat. No. 6,013,453; PCT WO 95/19431; PCT WO 98/54311 co-owned PCT/US00/00388 and references cited therein; co-owned U.S. patent application Ser. No. 09/444,241, filed Nov. 19, 1999; and co-owned U.S. patent application Ser. No. 09/535,088, filed Mar. 23, 2000. Methods for selection include, but are not limited to, phage display and in vivo selection.
>
> In methods comprising introduction of an exogenous molecule into a cell and testing for binding of the exogenous molecule to a binding site, a ZFP that binds to a target site, located within an accessible region, is designed. The designed ZFP can be introduced into the cell, or a

nucleic acid encoding the designed ZFP can be designed and the designed nucleic acid can be introduced into the cell, where it will express the designed ZFP. Methods for the design and/or selection of ZFPs that bind specific sequences are disclosed in U.S. Pat. No. 5,789,538; U.S. Pat. No. 6,007,408; U.S. Pat. No. 6,013,453; PCT WO 95/19431; PCT WO 98/54311 co-owned PCT/US00/00388 and references cited therein; co-owned U.S. patent application Ser. No. 09/444,241, filed Nov. 19, 1999; and co-owned U.S. patent application Ser. No. 09/535,088, filed Mar. 23, 2000. Methods for selection include, but are not limited to, phage display and in vivo selection.

In a preferred embodiment, an exogenous molecule is a zinc finger DNA-binding protein (ZFP). Certain ZFPs, their properties and their binding sequences are known in the art, as described supra. Furthermore, it is possible, for any particular nucleotide sequence, to design and/or select one or more ZFPs capable of binding to that sequence and to characterize the affinity and specificity of binding. See, for example, U.S. Pat. No. 5,789,538; U.S. Pat. No. 6,007,408; U.S. Pat. No. 6,013,453; PCT WO 95/19431; PCT WO 98/54311 co-owned PCT/US00/00388 and references cited therein; co-owned U.S. patent application Ser. No. 09/444,241, filed Nov. 19, 1999; and co-owned U.S. patent application Ser. No. 09/535,088, filed Mar. 23, 2000. Certain sequences, such as those that are G-rich, are preferred as ZFP binding sites. Since a **three**-finger ZFP generally binds to a 9- or 10-nucleotide target site, in a preferred embodiment, an accessible region, present within a region of interest in cellular chromatin, is searched for one or more G-rich sequences of 9-10 nucleotides and, for each sequence so detected, a ZFP can be designed to bind those sequences. In addition, two three finger modules can be joined, via an appropriate linker domain, to form a six-finger protein capable of recognizing an 18-20 nucleotide target site. See, for example, PCT/US99/04441.

Plasmids were constructed to encode transcriptional effector proteins containing zinc finger domains designed to recognize target sites surrounding the transcriptional initiation site of the human vascular endothelial growth factor (VEGF) gene; i.e. within the +1 accessible region described in Example 7. The target site has the sequence 5'-GGGGAGGATCGCGGAGGCTT-3'(SEQ ID NO: 1),

where the underlined T residue represents the major transcriptional startsite for the VEGF gene. A binding domain containing six zinc fingers, named VEGF 3a/1, was designed to bind to this 20-nucleotide target sequence. A three-finger zinc finger domain, VEGF 1 was designed to bind to the upstream 10-nucleotides of this target site having the sequence 5'-GGGGAGGATC-3' (SEQ ID NO: 2). A control six-finger domain, GATA 15.5, which was designed to bind the sequence 5'-GAGTGTGTGAACTGCGGGGCAA-3' (SEQ ID NO: 3), was also used. These zinc finger domains were encoded as fusion proteins in the NVF vector, as described below.

The zinc finger domains were constructed in a SP1 backbone. **The sequences of the recognition helices**, from position -1 to position +6, of VEGF 3a/1, VEGF 1 and GATA 15.5 are shown in Table 1. ...

The zinc finger domains contained **designed recognition helices**, as shown in Table 1, in a SP1 backbone.

An engineered fusion protein was designed to recognize a unique 9 base pair sequence in the DNase I hypersensitive region at –2 kb. This protein (BOS 3) comprised a nuclear localization sequence, a zinc finger binding domain, a KRAB repression domain and a FLAG epitope. The zinc finger binding domain was targeted to the sequence GGGGAGGAG, (SEQ ID NO: 27) which is complementary to the sequence CTCCTCCCC (SEQ ID NO: 28) in the coding strand. **Zinc finger sequences (for amino acids –1 through +6 of the recognition helices)** were RSDNLTR (SEQ ID NO: 29), RSDNLTR (SEQ ID NO: 30) and RSDALTK (SEQ ID NO: 31). Construction of a plasmid encoding the fusion protein and determination of the binding affinity of the zinc finger binding domain for its target sequence were performed according to methods disclosed in co-owned PCT WO 00/41566 and WO 00/42219. The dissociation constant (Kd) was determined to be 3.5 pM.

Thus, the term "non-naturally occurring" as used in the context of zinc finger proteins clearly relates to designed or selected zinc fingers that do not comprise a naturally occurring recognition helix sequence. The recitation "non-naturally occurring

recognition helix" is not antithetical to its plain meaning, but, rather, completely in keeping with the art-recognized and specification-detailed definitions and examples.

In addition, contrary to the Examiner's assertion on page 14 of the Examiner's Answer, the Board in *Ex Parte Dewis,* did not limit their determination that the term "non-naturally occurring" would clearly be understood by the skilled artisan to mean something that does not exist or is not found in nature to 35 U.S.C. § 112, 2nd paragraph rejections. *See*, *Ex parte Dewis* (Appeal 2007-1610, decided September 4, 2007). In fact, the Board addressed claim construction of the term "naturally occurring" for determination of both 35 U.S.C. § 112, 1st paragraph, written description and definiteness.

The Examiner also errs in asserting that because the specification does not disclose each and every "non-natural" sequence, "it is a truism that until all zinc fingers are sequenced, allowing the artisan to screen them, there is simply no way to tell whether any particular sequence occurs in nature or not." (Examiner's Answer, page 16). The claims are clear – if a recognition helix region is naturally occurring, the complex does not fall within the scope of the claims. At any point in time, it is a simple and straightforward matter for one of skill in the art to determine what is or is not naturally-occurring; thereby determining the scope of the claims. Thus, for the reasons of record and reiterated herein, the skilled artisan would have no doubt as to the scope of the term "non-naturally occurring," with respect to zinc finger proteins as a whole and to their individual recognition helix regions, as referring to zinc finger proteins whose recognition helices are not found in nature (i.e., are designed and/or selected to bind to a particular target site).

The Examiner also errs in asserting that "until a generic structure is identified, or it is known, for the vast majority of zinc finger helices which occur, or a deductive set of non-natural sequences are disclosed, it cannot be possessed as a genera." (Examiner's Answer, page 17). As previously noted, the as-filed specification, clearly teaches how to design or select zinc finger proteins in which all recognition helices regions are non-naturally occurring. The generic structure of non-naturally occurring zinc finger proteins is identified – the protein includes recognition helix regions that do not occur in nature.

As for a "deductive set of non-natural sequences," the specification exemplifies various such proteins and satisfies the written description requirement by showing possession of art-recognized ways of identifying additional embodiments.

It remains the case that the written description requirement is satisfied if reading the disclosure in light of the knowledge possessed by the skilled artisan at the time of filing, for example as established by reference to patents and publications available to the public prior to the filing date of the application, shows possession of the claimed subject matter. See, e.g., *In re Lange*, 209 USPQ 288 (CCPA 1981); *Falkner v. Inglis*, 79 USPQ2d 1001 (Fed. Cir. 2006); *Capon v. Eshhar* 76 USPQ2d. 1078 (Fed. Cir. 2005). Moreover, although working examples of multiple representative species are also never required to show possession, the case on appeal includes such examples. *Id.* See, e.g., *In re Lange*, 209 USPQ 288 (CCPA 1981). Thus, the Examiner has not met his burden of establishing why a skilled artisan would not have recognized that the applicant was in possession of claimed invention at the time of filing. *Vas-Cath, Inc. v. Mahurkar*, 19 USPQ2d 1111 (Fed. Cir. 1991); *In re Wertheim,* 191 USPQ 90 (CCPA 1976).

For the reasons of record and reiterated herein, the **claimed** subject matter is fully described in the as-filed specification and no new matter has been added and Appellants were in possession of the claimed complexes at the time of filing. Accordingly, the rejection cannot be sustained.

**C. Claims 57, 68, 70 and 71 are not anticipated by Choo**

Claims 57, 68, 70 and 71 remain rejected under 35 U.S.C. § 102(e) as allegedly anticipated by U.S. Patent No. 6,013,453 (hereinafter "Choo"), which was alleged to disclose the making of a mutant 3-fingered zinc finger protein that binds to the "coding sequence for a specific ras mutation" in human cells. (Examiner's Answer, pages 8-9). It was also alleged that "absent reason to believe otherwise, this site occurs within the broad definition of a general region which is in some way sensitive to digestion with DNAseI." *Id.*

In response to Appellants' arguments that Choo cannot anticipate the pending claims because this reference does not necessarily and inevitably teach that their zinc

finger proteins form a complex with a region of chromosomal cellular chromatin that sensitive to DNAseI digestion, it was asserted that "Applicant misquotes Choo out of context. As was stated Choo specifically demonstrates by Experiment that cellular chromatin can be so-bound, by utilizing integrated genes, and the paragraphs following the quotation demonstrate that it can be bound, demonstrating it to be enabled." (Examiner's Answer, citing Example 3 of Choo).

Appellants have neither misquoted nor taken out of context Choo's teachings. It is well settled that in order to support an anticipation rejection, the Office must show that every limitation of the claim at issue must appear identically in a single reference. *In re Bond,* 910 F.2d 831, 832, 15 USPQ2d 1566, 1567 (Fed. Cir. 1990). Furthermore inherent anticipation cannot be established by probabilities or possibilities (see, *Continental Can Co. USA , Inc. v. Monsanto Co.,* 20 USPQ2d 1746, 1749 (Fed. Cir. 1991):

> Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient.

Thus, in the instant case, Choo must teach a complex in which an engineered zinc finger protein is bound to a region of chromosomal cellular chromatin that is sensitive to DNAseI digestion.

The rejection is premised on improper claim construction, namely the assertion that chromosomal cellular chromatin as claimed encompasses Choo's integrated reporter sequences. As clearly defined in the specification, chromosomal cellular chromatin is endogenous chromatin (see, page 1, lines 10-12; page 10, lines 7 to 17 of the as-filed specification):

> The present disclosure is in the field of gene regulation, specifically, regulation of an endogenous gene in a cell and methods of regulating an endogenous gene through binding of an exogenous molecule.
>
> Chromatin is the nucleoprotein structure comprising the cellular genome. Cellular chromatin comprises nucleic acid, primarily DNA, and protein, including histones and non-histone chromosomal proteins.

> A chromosome, as is known to one of skill in the art, is a chromatin complex comprising all or a portion of the genome of a cell. The genome of a cell is often characterized by its karyotype, which is the collection of all the chromosomes that comprise the genome of the cell. The genome of a cell can comprise one or more chromosomes.
>
> An episome is a replicating nucleic acid, nucleoprotein complex or other structure comprising a nucleic acid that is not part of the chromosomal karyotype of a cell. Examples of episomes include plasmids and certain viral genomes.

Thus, the claims require that the complex be between chromosomal cellular chromatin, which is necessarily endogenous, and a non-naturally occurring zinc finger protein.

Accordingly, Choo's teaching in Example 3 regarding a reporter sequence that is integrated into a chromosome is not relevant to the pending claims. As repeatedly noted, Example 3 of Choo describes binding of an engineered zinc finger protein to an integrated BCR-ABL construct, which Choo clearly states is not an endogenous target in chromosomal cellular chromatin, as claimed (Choo, col. 26, lines 52-56, emphasis added):

> **Facsimiles** of these rearranged genes act as dominant transforming oncogenes in cell culture (Daley et al., 1988) and transgenic mice (Heisterkamp et al., 1990 Nature 344, 251-253). Like their genomic counterparts, the cDNAs bear a unique nucleotide sequence at the fusion point of the BCR and c-ABL genes, which can be recognised at the DNA level by a site-specific DNA-binding protein. The present inventors have designed such a protein to recognise the unique fusion site in the p190 BCR-ABL c-DNA. **This fusion is obviously distinct from the breakpoints in the spontaneous genomic translocations, which are though to be variable among patients.** Although the design of such peptides has implications for cancer research, the primary aim here is to prove the principle of protein design, and to assess the feasibility of in vivo binding to chromosomal DNA in available model systems.

Thus, by its own terms, Choo clearly states that their zinc finger proteins are not binding to chromosomal cellular chromatin (as required by the claims), but rather to a facsimile construct that is "obviously distinct" from the endogenous sequences.

Accordingly, the assertion that Example 3 of Choo demonstrates binding to chromosomal cellular chromatin (which is necessarily endogenous) is without merit.

Simply put, Choo's protein is not binding to chromosomal cellular chromatin. There is nothing untrue or misleading about quoting Choo directly in stating that they acknowledged the difference between their integrated reporter sequences and the claimed endogenous cellular chromatin. Example 3 of Choo simply shows that zinc fingers can bind to integrated reporter sequences. It does not describe or demonstrate binding of a zinc finger protein to chromosomal cellular chromatin as claimed and, therefore, cannot anticipate the pending claims.

Furthermore, Choo also fails to explicitly or inherently teach the claim element of binding to an endogenous chromosomal site that is sensitive to digestion with DNAseI. Choo clearly fails to explicitly disclose this limitation -- the terms "DNAseI" and "accessible regions" do not occur anywhere in this reference. Nor does Choo implicitly (*i.e.,* necessarily and inevitably) teach their integrated sequence is in a region sensitive to DNAseI digestion.

Therefore, Choo does not describe or demonstrate complexes of zinc finger binding proteins with cellular chromosomal chromatin, as claimed. As such, Choo cannot anticipate any of the pending claims and the rejection cannot be sustained.

**D. Claims 57 and 68-71 are not obvious over the cited references**

Claims 57 and 68-71 also remain rejected under 35 U.S.C. § 103(a) as allegedly obvious over Choo in view of WO 00/9837755 (hereinafter "Dangl"). (Examiner's Answer, pages 9-10). Choo was cited as above. While it was acknowledged that Choo does not teach plant cells, Dangl was cited for teaching that zinc finger proteins function in plant cells. *Id.*

In response to Appellants' arguments that showing binding of a zinc finger protein to a randomly integrated sequence is not the same as (or predictive of) binding to endogenous chromosomal cellular chromatin, as claimed, it was asserted that Choo's randomly integrated sequence is a chromosomal sequence as claimed and Example 3 of

Choo "demonstrates that binding" to this randomly integrated sequences is not a problem. (Examiner's Answer, page 20).

Furthermore, in response to the additional evidence or record (Beerli and Borman) establishing the differences between binding to chromosomal cellular chromatin (as claimed) and a randomly integrated sequence (Choo), the Examiner stated the following (Examiner's Answer, page 21-22, emphasis added):

> ...Borman and Beerli are papers comprising authors who were inventors in the Choo patent.[1] They do specifically state that they performed, for the first time, binding to natural sites, in natural chromatin context, in cells, after the priority date of Appellant. They do state that prior to their demonstration, "willful and specific regulation of endogenous genes with designed transcription factors has remained an unmet challenge in biology." However, there is no scientific basis to believe it would not work prior to an actual demonstration. There is no reason to know why it remained an unmet challenge in biology. Simply put, at best, it appears to the examiner that Borman and Beerli and Choo, were busy with other things, and this is the first time they decided to prove it. They provide no rationale as to why it might not work...

The Examiner's assertions are completely untenable. Borman, Beerli and Choo provide clear rationales as to why binding to endogenous chromosomal cellular chromatin was unpredictable from Choo's experiments on randomly integrated reporter sequences. As reiterated above, Choo clearly states that their integrated reporter is "distinct" from endogenous sequences. *See, also,* Beerli et al. (2000) *Proc. Natl. Acad. Sci. USA* 97:1495-1500, page 1496, left column, reference AH-1 of IDS submitted May 3, 2002 and Evidence Appendix (3), emphasis added:

> We have previously described the generation of designed transcription factors capable of specifically regulating an *erbB-2* promoter-luciferase construct (12). Here we target endogenous *erbB-2* gene for imposed regulation ... **Endogenous genes are packaged within chromatin and are controlled by a multiplicity of *cis*- and *trans*-acting factors** (22, 23), **making it not known *a priori* whether specific gene regulation imposed with a dominant designed transcription factor is possible.**

[1] Appellants note that Borman and/or Beerli are not co-authored by the present inventors but from a different group working the zinc finger protein field.

Thus, contrary to the Examiner's assertion, the skilled artisan, including Choo, did not believe binding to endogenous cellular chromatin was predictable from reporter construct, for example because of chromatin structure found in chromosomal cellular chromatin.

Likewise, the assertion that the skilled artisan working in this area was too "busy with other things," to try binding to endogenous targets is entirely nonsensical. Endogenous gene regulation was the goal of all groups working in this area – not something they were "too busy" to try. Indeed, it is plain that had Choo thought it possible to regulate an endogenous cellular gene, they would have selected an engineered zinc finger protein that bound to a target sequence in an endogenous cellular gene, rather than the zinc finger protein actually selected, which binds to a breakpoint junction sequence in a cDNA. Furthermore, after selecting a zinc finger protein to bind to the non-endogenous target, the non-endogenous fusion cDNA had to be introduced into cells and cells selected that had randomly integrated the non-endogenous cDNA into their chromosomes. Only after all of these manipulations had been performed could the ZFP be tested. The additional time and effort expended to construct, transfect and attempt to bind to a non-endogenous target (which Choo states is a "facsimile" that is "obviously distinct" from endogenous sequences) indicates that it was considered completely **unpredictable by the skilled artisan** whether engineered zinc finger proteins would bind to chromosomal cellular chromatin. Simply put, there is no description or suggestion to use of an engineered zinc finger protein to bind to chromosomal cellular chromatin that is sensitive to DNAseI digestion as claimed...

Thus, Choo does not teach or suggest binding of a zinc finger protein to endogenous chromosomal cellular chromatin as claimed and the Examiner's contention that this was because they were "too busy" to actually do a much simpler experiment than the one they did is without merit. Clearly, the skilled artisan working in this field is on the record as stating that binding of engineered zinc finger proteins to chromosomal cellular chromatin was <u>not</u> predictable based on Choo's disclosure. As such, Choo does not teach the claimed elements or in any way suggest their predictability.

Therefore, there is no combination of Choo and Dangl that render any of the pending claims obvious over these references and the rejection must be withdrawn.

## CONCLUSION

For the reasons set forth herein, allowance of the claims under consideration is requested.

Respectfully submitted,

Date: Sept 28, 2010

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## CLAIMS APPENDIX

**57.** A cell comprising a complex between

a non-naturally occurring zinc finger protein comprising 3 or more zinc finger domains, wherein the zinc finger domains comprise a non-naturally occurring recognition helix and

chromosomal cellular chromatin;

wherein the zinc finger protein is bound to a target site in a region of the cellular chromatin that is sensitive to digestion with DNAseI.

**68.** The cell of claim 57, wherein the zinc finger protein is encoded by a nucleic acid introduced into the cell.

**69.** The cell of claim 57, wherein the cell is a plant cell.

**70.** The cell of claim 57, wherein the cell is an animal cell.

**71.** The cell of claim 57, wherein the cell is a human cell.

## EVIDENCE APPENDIX

The following documents were submitted with the Appeal Brief and, therefore, are not re-submitted herewith:

(1) U.S. Patent No. 5,789,538, cited reference AA-1 of IDS submitted May 3, 2002, indicated considered by the Office on July 19, 2004;

(2) U.S. Patent No. 6,013,453, cited as reference AC-1 of IDS submitted on May 3, 2002, indicated considered by the Office on July 19, 2004;

(3) Beerli et al. (2000) *Proc. Nat'l. Acad. Sci. USA* 97:1495-1496, cited as reference AH-1 of IDS submitted on May 3, 2002, indicated considered by the Office on July 19, 2004; and

(4) Borman et al. (2000) "DNA-Binding Proteins Turn Genes On and Off." C&CEN, submitted with the Response After Final dated June 10, 2009.

## RELATED APPEALS AND INTEFERENCES

As noted above, Appellants are not aware of any additional appeals or interferences other than the ones identified in the Appeal Brief.